Effect of Modification of Thyroid Function on Cholesterol 7 α -Hydroxylation in Rat Liver

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Hepatic activities of cholesterol synthesis and cholesterol 7 α -hydroxylation were determined in hyper- and hypo-thyroid rats after oral administration of glucose or cholesterol. Increases in activities of both cholesterol synthesis and cholesterol 7 α -hydroxylation induced by glucose administration were enhanced by pretreatment with thyroid powder but suppressed by pretreatment with thiouracil. The enhancement of 7 α -hydroxylation was produced by a relatively small amount of thyroid powder, but high doses were required to increase cholesterol synthesis. On the other hand, the suppression of 7 α -hydroxylation was brought about by a low dose of thiouracil, but high doses were required to decrease cholesterol synthesis. Although cholesterol synthesis increased similarly in both hypo- and hyper-thyroid rats after glucose administration, hydroxylase activity in hypothyroid rats began to increase more slowly and was always lower than that in hyperthyroid rats. Thus it is concluded that cholesterol 7 α -hydroxylase activity is more sensitive to thyroid function than are activities of cholesterol-synthetic enzymes. When exogenous cholesterol was given, hypothyroid rats showed a larger increase in serum cholesterol concentration than hyperthyroid rats, and there was an inverse relationship between serum cholesterol concentrations and hepatic cholesterol 7 α -hydroxylase activities.

Our previous report showed that the activity of cholesterol 7 α -hydroxylation in rat liver is closely related to that of hepatic cholesterol synthesis (Takeuchi *et al.*, 1974). The increase in hepatic cholesterol synthesis which occurs after oral glucose administration is followed by an increase in cholesterol hydroxylation with a lag phase of about 1 h. Stimulation of hepatic cholesterol synthesis by bile-duct ligation also induces an increase in hepatic cholesterol hydroxylation (Danielsson, 1973; Takeuchi *et al.*, 1974), but occurs more slowly than the increase after glucose administration. Thus it is suggested that a good correlation exists between both activities in general. However, a disagreement is also observed in some circumstances. The treatment with Triparanol 2-(*p*-chlorophenyl){1-[4-(diethylaminoethoxy)phenyl]-1-(*p*-tolyl)ethanol}, which decreases serum cholesterol concentration, markedly decreased hepatic cholesterogenesis without affecting 7 α -hydroxylation activity (Takeuchi *et al.*, 1974).

It is well known that thyroid hormone profoundly affects cholesterol metabolism. Patients with hyperthyroidism have low serum cholesterol concentrations, whereas those with hypothyroidism show high concentrations. The turnover rate of cholesterol in hyperthyroidism was proved to be faster than that in hypothyroidism (Rosenman *et al.*, 1952; Weiss & Marx, 1955; Lepp *et al.*, 1964).

In this connexion, the responses of cholesterol synthesis and cholesterol 7 α -hydroxylation to oral glucose administration have now been examined in rats previously treated with various doses of thyroid powder or thiouracil.

Materials and Methods

Female Sprague-Dawley rats weighing 180–200 g were used. They were fed on a balanced stock diet (Oriental Kobo Ltd., Osaka, Japan) and water *ad libitum*. Daily, 6, 18 or 54 mg of desiccated thyroid powder per rat (Teikoku-zoki Ltd., Tokyo, Japan; 1 mg of thyroid powder is equivalent to 1 μ g of L-thyroxine) to make the hyperthyroid state, or 50, 75 or 100 mg of 2-thiouracil per rat to make the hypothyroid state were administered for 10 days by means of a stomach tube. Control rats were given vehicle water alone. After the rats had been starved for 36–40 h, 2 ml of 50% (w/v) glucose solution per 100 g body wt. was given by stomach tube, then animals were killed by decapitation 3 h later. During the starvation, the administration of thyroid powder or thiouracil was carried out during the evening before the day of glucose ingestion. To follow the changes in hepatic activities of cholesterol synthesis and cholesterol hydroxylation as a function of time, both groups of rats, given either 6 mg of desiccated thyroid

powder or 50 mg of thiouracil for 10 days, were killed 30 min, 1, 2, 3 or 4 h after glucose administration. Paired control rats of both groups were killed immediately after the ingestion of vehicle water.

Cholesterol suspension in peanut oil was made by mixing 1.3 g of cholesterol and 0.3 g of cholic acid in 40 ml of peanut oil. This suspension (2 ml/100 g body wt.) was given at 10:00 h for 3 days to rats that had previously been given, daily, either 10 g of the standard diet supplemented with 0.06 or 0.18% of desiccated thyroid powder or 0.5 or 0.75% of thiouracil for 7 days. During these 3 days of cholesterol administration, rats were given the stock diet and received 6 mg or 18 mg of thyroid powder, or 50 or 75 mg of thiouracil, by stomach tube, because dietary consumptions were decreased by cholesterol administration.

Lipogenesis and cholesterologenesis from acetate, and cholesterol 7 α -hydroxylation activity in the liver, were determined *in vitro* as reported previously (Takeuchi *et al.*, 1974). Serum cholesterol concentration was determined by the method described by Leffler & MacDougald (1963). Statistical significances were estimated by Student's *t* test.

Results

Thyroid powder generally decreased serum cholesterol concentration, but the response was not dependent on the dose. The lowest concentration was obtained in rats given 18 mg of thyroid powder, which showed about 20% decrease compared with controls ($P < 0.02$), but no statistically significant decrease was found in rats given 6 mg or 54 mg of thyroid powder.

Thiouracil, by contrast, increased serum cholesterol concentration. A significant increase of about 20% was found in rats given 50 mg of thiouracil, but increases in rats given higher doses were statistically insignificant on account of relatively large variations of the cholesterol concentration. Rats given 100 mg of thiouracil showed decreased food intake, to about half that of the controls, and lost 10–15% of their body weight. This may relate to the large variations of serum cholesterol concentrations.

It was concluded that serum cholesterol concentration in hyperthyroid rats was lower than that of hypothyroid rats (54.2 ± 2.15 versus 72.5 ± 2.26 mg/100 ml, $P < 0.001$). When compared with values of the individual groups in both hyper- and hypothyroid rats given different doses of the drugs, there were significant differences between hyper- and hypo-thyroid rats except in some cases shown in Table 1.

Hepatic lipogenesis *in vitro* and cholesterologenesis and cholesterol 7 α -hydroxylation 3 h after glucose administration in hyper- and hypo-thyroid rats are presented in Figs. 1 and 2 respectively. There was no

Table 1. Serum cholesterol concentrations of rats treated with various amounts of thyroid powder or thiouracil

Results are means $\pm$ S.E.M., for the numbers of rats given in parentheses. Statistical significance was determined by Student's *t* test. Abbreviation: ns, not significant ($P > 0.05$).

(a) Animal groups	Serum cholesterol (mg/100 ml)
Control (11)	60.8 ± 2.88
Hyperthyroid rats	
Total (31)	54.2 ± 2.15
Thyroid powder, 6 mg (13)	54.5 ± 3.66
Thyroid powder, 18 mg (9)	49.4 ± 3.22
Thyroid powder, 54 mg (9)	58.6 ± 3.89
Hypothyroid rats	
Total (16)	72.5 ± 2.26
Thiouracil, 50 mg (10)	73.9 ± 3.00
Thiouracil, 75 mg (3)	69.8 ± 6.02
Thiouracil, 100 mg (3)	70.0 ± 5.03
(b) Statistical significances between two groups	
Control versus hyper (total)	ns
Control versus hypo (total)	0.01
Hyper (total) versus hypo (total)	0.001
Control versus hyper (6 mg)	ns
Control versus hyper (18 mg)	0.02
Control versus hyper (54 mg)	ns
Control versus hypo (50 mg)	0.01
Control versus hypo (75 mg)	ns
Control versus hypo (100 mg)	ns
Hyper (6 mg) versus hypo (50 mg)	0.001
Hyper (6 mg) versus hypo (75 mg)	ns
Hyper (6 mg) versus hypo (100 mg)	0.05
Hyper (18 mg) versus hypo (50 mg)	0.001
Hyper (18 mg) versus hypo (75 mg)	0.02
Hyper (18 mg) versus hypo (100 mg)	0.01
Hyper (54 mg) versus hypo (50 mg)	0.05
Hyper (54 mg) versus hypo (75 mg)	ns
Hyper (54 mg) versus hypo (100 mg)	ns

significant difference between the incorporations of acetate into total lipids and cholesterol *in vitro* in the livers of the rats treated with two lower doses of both thyroid powder and thiouracil. However, the highest dose of thyroid powder markedly increased both lipogenesis and cholesterologenesis, as shown in Fig. 1, whereas the highest dose of thiouracil markedly decreased them, as shown in Fig. 2.

The increase in hepatic cholesterol 7 α -hydroxylation after glucose administration was significantly enhanced in the rats treated daily with 6 mg of thyroid powder, the lowest dose, and further enhanced by the treatment with higher doses (Fig. 1). The highest dose, 54 mg daily, caused about a 3-fold increase. With regard to thyroid powder, a dose-dependent enhancement of 7 α -hydroxylation activity was observed. However, with daily treatment, 50 mg of thiouracil decreased cholesterol 7 α -hydroxylation activity, and increasing the doses up to 100 mg did not

Fig. 1. Synthesis of hepatic total lipid and cholesterol and cholesterol 7 α -hydroxylation in rats treated with thyroid powder

Rats were treated with 6, 18 or 54mg of thyroid powder daily for 10 days. After starvation for 36–40h, 4ml of 50% (w/v) glucose was intubated by stomach tube and the animals were killed after 3h. Lipogenesis and cholesterogenesis from acetate and cholesterol 7 α -hydroxylation were determined *in vitro*. For details, see the Materials and Methods section. Numbers of rats are given in parentheses.

further influence the lower rate of hydroxylation, as shown in Fig. 2. It is noteworthy that cholesterol 7 α -hydroxylation activity is increased by doses such as 6mg or 18mg of thyroid powder which were not enough to cause an increase in cholesterol synthesis (Fig. 1), and that the activity was decreased by doses such as 50 or 75mg of thiouracil, which were not enough to cause a decrease in cholesterol synthesis (Fig. 2).

Fig. 3 shows the hourly changes in the activities of cholesterol synthesis and 7 α -hydroxylation after glucose administration in both groups of rats treated daily with either 6mg of thyroid powder or 50mg of thiouracil for 10 days. Rats were starved for 36h before glucose ingestion, as mentioned above. The activities of cholesterol synthesis in both groups were almost identical at 0h. They began to increase 1h after glucose administration and showed maximum responses at 3–4h. Although hypothyroid rats seemed to show lower values, there were no statistically significant differences in cholesterol synthesis

Fig. 2. Synthesis of hepatic total lipid and cholesterol and cholesterol 7 α -hydroxylation in rats treated with thiouracil

Rats were treated with 50, 75 or 100mg of thiouracil daily for 10 days. Further details were as in Fig. 1.

between both groups of rats except the results at 4h after glucose administration.

On the other hand, cholesterol 7 α -hydroxylase activities in hyper- and hypo-thyroid rats were similar at 0h, as shown in Fig. 3. In hyperthyroid rats, hydroxylase activity quickly responded, increasing as early as 30min after glucose administration, by about 2-fold by 1h, and remaining high at 4h. In hypothyroid rats, the activity slightly increased 2 and 3h after glucose administration but returned to the initial low concentration at 4h. Thus the activity of hydroxylation was always lower in hypothyroid rats than in hyperthyroid rats, and the differences were statistically significant. This suggests that the activities of cholesterol synthesis and 7 α -hydroxylation do not change in parallel and that hydroxylase activity is more sensitive to thyroid function than is cholesterogenesis.

Table 2 shows serum cholesterol concentrations and hepatic cholesterol 7 α -hydroxylase activity in hyper- and hypo-thyroid rats which had previously ingested a large amount of cholesterol in peanut oil. The serum cholesterol concentration of hypothyroid rats was markedly increased by cholesterol intubation and was significantly higher than that of hyperthyroid rats. The serum cholesterol concentration of the rats

Fig. 3. Cholesterol synthesis and cholesterol 7α -hydroxylation at various time-intervals in hyper- and hypo-thyroid rats after glucose ingestion

Rats were given 6mg of thyroid powder or 50mg of thiouracil for 10 days. After starvation for 36–40h, 4ml of 50% glucose (w/v) solution was intubated. After 30min, 1, 2, 3 or 4h, the rats were killed. Cholesterol synthesis from acetate and cholesterol 7α -hydroxylation were determined *in vitro*. —, Hyperthyroid rats; ----, hypothyroid rats. The asterisks represent the statistical significances to 0h controls. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig. 4. Relationship between serum cholesterol and hepatic cholesterol 7α -hydroxylase activity in hyperthyroid rats given cholesterol suspension orally for 3 days

Cholesterol suspension in peanut oil was made by mixing 1.3g of cholesterol and 0.3g of cholic acid in 40ml of peanut oil. This suspension (2ml/100g body wt.) was intubated for 3 days to hyper- and hypo-thyroid rats previously fed daily with 10g of the stock diet containing 0.06 or 0.18% of desiccated thyroid powder. During these 3 days of cholesterol administration, rats were given the stock diet and received 6mg or 18mg of thyroid powder by stomach tube. Hepatic cholesterol 7α -hydroxylation was determined *in vitro*. ○, Values for the rats treated with 18mg of thyroid powder; ●, values for rats treated with 6mg of thyroid powder. r (correlation coefficient) = 0.83 ($P < 0.01$); y (regression line) = $-1.80x + 1060$.

Table 2. Serum cholesterol and cholesterol 7α -hydroxylation of the hyper- and hypo-thyroid rats intubated with the cholesterol suspension

Results are means $\pm$ S.E.M., for the numbers of rats given in parentheses. Statistical significance was determined by Student's t test. Abbreviation: ns, not significant ($P > 0.05$).

	Serum cholesterol (mg/100ml)	Cholesterol 7α -hydroxylation (nmol/h per mg of protein)
(a) Animal groups		
Control (5)	150.9 $\pm$ 20.4	0.63 $\pm$ 0.02
Hyperthyroid rats		
Total (9)	112.7 $\pm$ 28.3	0.84 $\pm$ 0.06
Thyroid powder 6mg (4)	179.5 $\pm$ 34.8	0.71 $\pm$ 0.05
Thyroid powder 18mg (5)	61.4 $\pm$ 5.6	0.94 $\pm$ 0.07
Hypothyroid rats		
Total (14)	195.0 $\pm$ 21.0	0.32 $\pm$ 0.03
Thiouracil 50mg (9)	190.0 $\pm$ 27.9	0.31 $\pm$ 0.03
Thiouracil 75mg (5)	204.6 $\pm$ 34.1	0.35 $\pm$ 0.04
(b) Statistical significances between groups		
Control versus hyper (total)	ns	<0.05
Control versus hypo (total)	ns	<0.001
Hyper (total) versus hypo (total)	<0.05	<0.001
Hyper (6mg) versus hyper (18mg)	<0.01	<0.05
Hypo (50mg) versus hypo (75mg)	ns	ns
Hyper (6mg) versus hypo (50mg)	ns	<0.001
Hyper (6mg) versus hypo (75mg)	ns	<0.001
Hyper (18mg) versus hypo (50mg)	<0.001	<0.001
Hyper (18mg) versus hypo (75mg)	<0.001	<0.001

given 18mg of thyroid powder daily was hardly increased by cholesterol administration. Hepatic cholesterol 7 α -hydroxylase activity was lower in hypothyroid rats than in hyperthyroid rats.

The relationship between serum cholesterol concentration and hepatic cholesterol 7 α -hydroxylation activity in hyperthyroid rats is shown in Fig. 4. A statistically significant correlation ($r = -0.83$, $P < 0.01$) was found. Such a correlation, however, was not found in hypothyroid rats.

Discussion

Our previous report (Takeuchi *et al.*, 1974) indicated that the increase in cholesterol 7 α -hydroxylation was induced successively by the increase in hepatic cholesterol synthesis in the liver. The serum cholesterol concentration, however, remained fairly constant even if hepatic cholesterol synthesis was markedly enhanced by glucose ingestion, probably because cholesterol-catabolic reactions were also stimulated with a slight delay after the increase in cholesterol synthesis. It is well documented that β -hydroxy- β -methylglutaryl-CoA reductase, which is a rate-limiting enzyme of cholesterol synthesis, shows diurnal variation (Back *et al.*, 1969; Shapiro & Rodwell, 1969; Dugan *et al.*, 1972). However, serum cholesterol concentration scarcely changes during 24h, as demonstrated in the studies of Page & Moinuddin (1962) and Hollister & Wright (1965). Danielsson (1972) has reported that the activity of hepatic cholesterol 7 α -hydroxylation also fluctuates in parallel with the change of β -hydroxy- β -methylglutaryl-CoA reductase. So this regulation mechanism of cholesterol by both enzymes has to maintain the homeostasis of serum cholesterol concentration (Takeuchi *et al.*, 1974).

The present experiments demonstrated that activities of both cholesterol synthesis and cholesterol 7 α -hydroxylation were increased by desiccated thyroid powder and decreased by thiouracil. In addition, they demonstrated that cholesterol 7 α -hydroxylation was accelerated by low doses of thyroid hormone which caused no increase in cholesterol synthesis, and was suppressed by doses of thiouracil which did not decrease cholesterol synthesis. From these results, it is concluded that cholesterol 7 α -hydroxylation is more susceptible to thyroid function than is cholesterol synthesis. This conclusion is reconcilable with the supposition that thyroxine increases cholesterologenesis but further increases cholesterol catabolism (Rosenman *et al.*, 1952; Weiss & Marx, 1955; Lepp *et al.*, 1964).

Fletcher & Myant (1960) reported that cholesterol synthesis from acetate was increased by the injection of 20 μ g of thyroxine but decreased by 30 or 50 μ g. However, we found an increase in cholesterologenesis in rats given 54mg of thyroid powder, which was equivalent to 54 μ g of L-thyroxine. This discrepancy

may relate to a difference in experimental conditions. The drug was administered orally and the experiments were performed under the condition that cholesterologenesis was maximally stimulated by glucose ingestion in our experiments.

The serum cholesterol concentration in hyperthyroid rats was lower than that in the normal controls, but the difference was not statistically significant. Only the rats given 18mg of thyroid powder showed a statistically significant decrease in serum cholesterol concentration. This may be the reason that the dose of 18mg of thyroid powder markedly stimulated the hydroxylation without affecting cholesterologenesis.

As shown in Fig. 3, cholesterol synthesis increased 1 h after glucose administration. In hyperthyroid rats, however, the increase in hydroxylation began as early as 30min after glucose ingestion. It is suggested that thyroid hormone stimulates the catabolic reaction of cholesterol to bile acid primarily. Eskelson (1968) reported that the increase in cholesterol synthesis by thyroid hormone was derived from stimulation of cholesterol catabolism secondarily.

The activity of 7 α -hydroxylation in hypothyroid rats was lower than that in hyperthyroid rats throughout the period of the experiment and the manifestation of the increase in cholesterol hydroxylation in hypothyroid rats was delayed when compared with hyperthyroid rats. This suggests a decrease in enzyme induction of cholesterol hydroxylation in hypothyroid rats. Therefore the response of serum cholesterol concentration to dietary cholesterol was higher in hypothyroid rats than in hyperthyroid rats.

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